

INDUCTION OF MUTATIONS IN A BACTERIAL VIRUS*

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Introduction.—In the course of experiments designed for other purposes a paradoxical observation was made: phage λ , inactivated by UV irradiation, when adsorbed onto sensitive bacteria was reactivated when a further dose of UV was given to the phage-bacterium complexes. Among the reactivated phages a fairly large proportion were mutants. A description of these findings and a discussion of their implications will be found below.

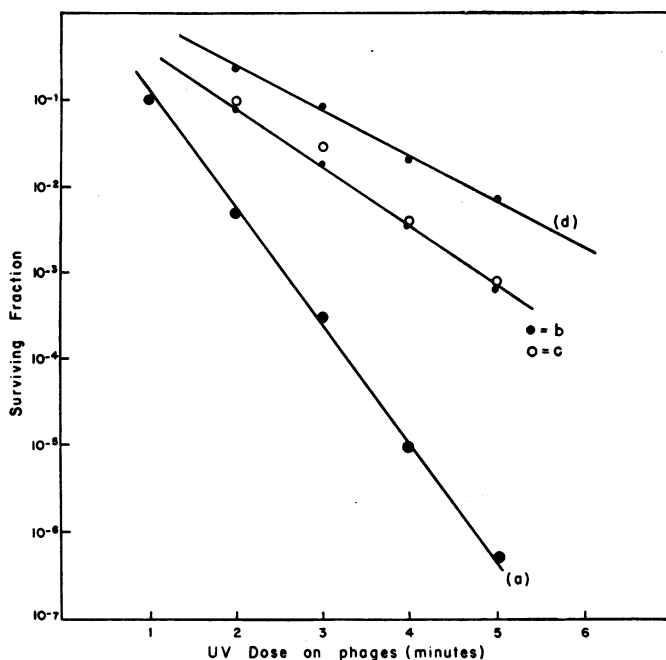


FIGURE 1

Semilogarithmic plots of the UV surviving fraction of irradiated phage λ plated (a) on non-irradiated bacteria, (b) on irradiated bacteria (60 sec., 10^{-1} survival).

Material and Methods.—Bacteria: *E. coli* strain K12 (λ), lysogenic for the temperate phage λ , and different derivatives called K12S, having lost the lysogenic character and having become thus sensitive to λ ; *E. coli* strain C (Bertani and Weigle¹). Phages: The temperate phages liberated by K12 (λ) and mutants of these phages, differing in the morphology of their plaques or in virulence.

The general methods used were those described by Adams.²

Ultra-violet irradiation of suspensions in buffer of bacteria or phages was done at a distance of 37 cm. from a 15-watt Sterilamp. At that distance, 8 sec. of irradiation leave a surviving fraction of 10^{-2} of the phage T2.

For visible light illumination, the plates were exposed at room temperature to the light of two parallel fluorescent lamps of 40 watts each at a distance of 30 cm. In a few experiments a General Electric H5 lamp of 250 watts was used (Dulbecco³).

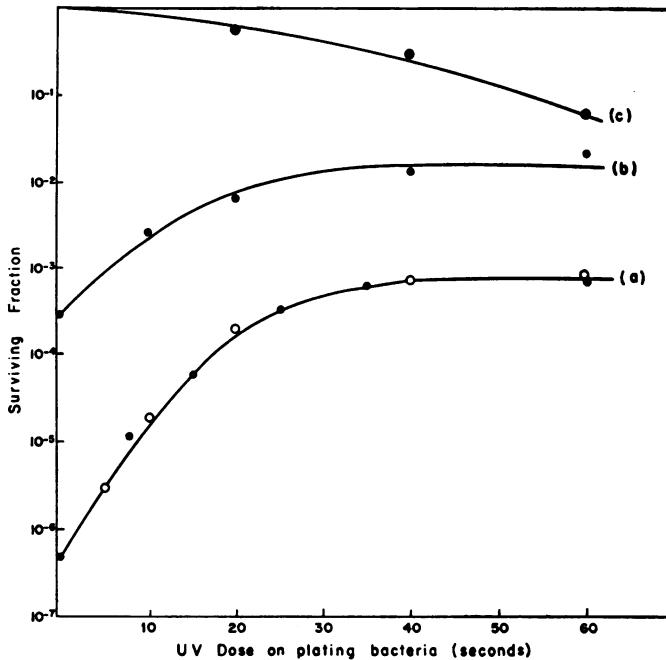


FIGURE 2

Semilogarithmic plots of the surviving fraction of λ phages having received a dose of (a) 5 min. UV irradiation (2 experiments), (b) 3 min. UV irradiation, as a function of the dose of UV given to the plating bacteria. The upper curve (c) shows the surviving fraction (as colony formers) of the irradiated bacteria.

Reactivation by UV'd Bacteria of Inactivated Phages.—Phage λ irradiated with UV and plated on *E. coli* strain K12S has a survival curve approximating a three-hit curve (Fig. 1, curve a). If the UV'd phages are plated on UV'd bacteria (dose of 60 sec., 10^{-1} survival as colony formers) the survival curve is still approximately a three-hit curve but the surviving fraction is much larger (Fig. 1, curve b).

The reactivation due to the UV'd bacteria depends on the dose of UV

received by the bacteria (Fig. 2). At low doses the reactivation increases with the dose. Saturation is reached at doses larger than 50 sec.; half of the reactivation is accomplished at a dose of 25 sec.

Reactivation can be obtained by irradiation of the bacteria either before adsorption of the inactivated phages to the bacteria or afterward (in the latter case the supplementary dose of UV given to the phages must be taken into account). If the complexes (UV'd phage-normal bacterium) are kept at room temperature in nutrient agar before irradiation, their reactivable half-life time is approximately 30 minutes.

If the irradiation of the bacteria has taken place before adsorption of the phages, the bacteria retain their reactivating ability for many days when kept in buffer at 4°C. The irradiated phages also retain for days their ability to be reactivated.

For non-irradiated phages the plating efficiency on irradiated bacteria is the same as that on normal bacteria.

The progeny of the reactivated phages has the same efficiency of plating on normal as on irradiated bacteria.

The UV-inactivated phages do not kill the bacteria. Even the reactivable particles do not kill the bacteria on which they are adsorbed.

The λ phages do not show multiplicity reactivation with the exception of the virulent mutant (Jacob⁴).

Reactivation (and the production of mutants to be described below) can be obtained not only by exposure of the bacteria to UV but also by their exposure to x-rays or to Dichlorene⁵ (nitrogen mustard). These last two agents are known⁶ to induce lysis in lysogenic bacteria as effectively as UV. Treatment of the bacteria with H₂O₂ does not produce reactivation (or mutations).

The treatment of the irradiated phages or of the non-irradiated bacteria or of both with the supernatant of an irradiated bacterial suspension does not produce reactivation. Neither does adsorption of UV'd λ on non-irradiated bacteria followed by plating on UV'd bacteria. The adsorption of the inactivated phage to the UV'd bacteria is thus necessary for reactivation to occur.

All the different mutants of λ have the same UV sensitivity. They are all reactivable by UV'd bacteria but the maximum amount of reactivation obtainable is slightly different for each of them.

All the mutant strains of the sensitive bacteria tested, differing in their nutritional requirements or having the F⁺ or F⁻ character (see Hayes;⁷ Cavalli, Lederberg, and Lederberg⁸) give reactivation (and mutations). *E. coli* strain C also produces both.

The reactivation by irradiated bacteria does not take place for UV inactivated T2, T3, or T5 (only T's tested) if plated either on UV'd strain B or UV'd strain K12S.

Appearance of Mutants Among the Reactivated Phages.—Mutants, producing plaques of strikingly different morphologies, are found among the reactivated phages. The parent type forms fairly turbid plaques because of the production of lysogenic bacteria which are resistant to the phage (Fig. 3). Six different types of mutants can easily be distinguished. Some give clear plaques, others give plaques which are more turbid than those of the parent type. Some of the mutants are unstable, giving rise, on subculture, to other mutants. With the exception of a few per cent, each plaque formed by a reactivated λ , showing a morphology different from that of the parent, contains only one type of phage.

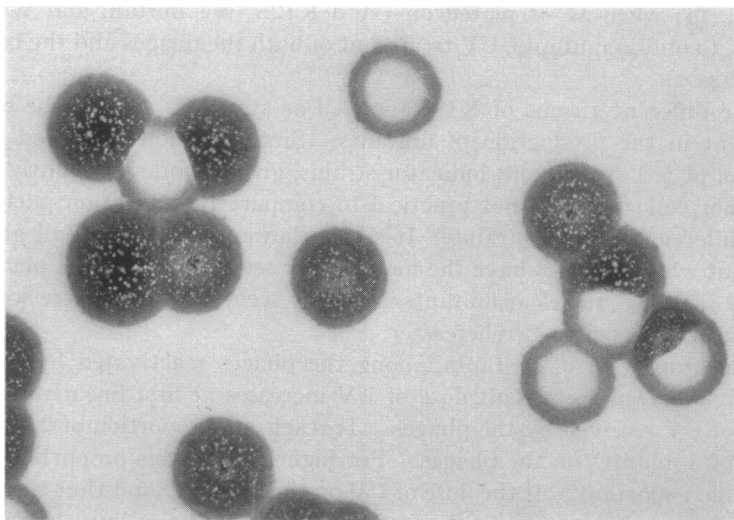


FIGURE 3

Plaques of the parent λ phage and of two different clear plaque-forming mutants, on 60-sec. irradiated bacteria. The parent produces turbid plaques; one of the clear mutant shows no growth in the center of the plaque while the other one does.

The mutants plate on normal and on irradiated bacteria with the same efficiency. They all have the same UV sensitivity and the same latent period as the parent. Their burst sizes, however, are different. They vary greatly from one another, and from the parent, in the efficiency with which they produce lysogenization of the bacteria they infect (this may be the main cause of the differences in the morphologies of their plaques). The mutants seem to be inactivated by heat at different rates.

When the mutants are inactivated with UV and reactivated by UV'd bacteria they, in turn, usually give rise to other mutants. Each mutant seems to have a characteristic mutation pattern. No spontaneous or in-

duced reversion of a mutant to the parent type has been observed. No host range mutant has been found. The virulent mutant although reactivable does not show any plaque morphology mutants nor does it give rise to host range mutants.

Some of the mutants appear spontaneously in the cultures of the parent phage with frequencies which are at most of the order of 0.05%. Mutants are also present in the lysate of lysogenic cultures induced with very high doses of UV.

No mutants are found (1) among the survivors of UV'd λ plated on normal bacteria, (2) when non-irradiated λ is plated on UV'd bacteria, (3) when UV'd λ is plated on non-irradiated bacteria and is photoreactivated, (4) when λ C is plated on UV'd K12S (see Bertani and Weigle¹). Thus, to obtain mutants, UV treatment of both the phages and the bacteria is necessary.

The different strains of K12S (as well as strain C) seem to be equally efficient in the production of mutants. Since the non-irradiated parent phage, plated on different indicator strains, gives plaques of slightly different morphologies, it is not practical to compare the mutation pattern on the different sensitive strains. It seems, however, that they all give the mutant whose plaques have the morphology seen in the clearest plaques of figure 3. Since the clear mutants are easily seen, only these were scored in the experiments described hereafter.

The proportion of mutants among the phages reactivated by bacteria having received a constant dose of UV increases at first linearly with the dose of UV received by the phages. It reaches a proportion of 2.5% for a dose of 5 minutes on the phages. For higher doses, this proportion seems to remain constant. If the dose of UV on the bacteria (and thus the reactivation) is varied, the proportion of mutants also varies linearly with the bacterial dose, reaching the maximum value of 2.5% for maximum reactivation (60 sec. of UV on the bacteria).

Photorestitution.—(a) Photorestitution of the UV'd bacteria—UV'd phages, plated on UV'd bacteria which have been illuminated with visible light, are not reactivated and show no mutants. The dose of light necessary to produce this effect is large and increases with the dose of UV received by the bacteria (one hour exposure to a G. E. H5 lamp is necessary to restore 15 sec. UV'd bacteria).

(b) Photorestitution of the complexes (UV'd phage-non-irradiated bacterium).—The dose of visible light necessary for maximum restoration of the (λ -strain K12S) complexes is approximately the same as that necessary for the restoration of (T2-strain B) complexes. The maximum amount of photorestitution is of the same order of magnitude as that obtained by plating on UV'd bacteria. When complexes are formed with different bacterial strains, the amount of photorestitution of one strain

does not differ by a factor larger than 10 from that of another strain (for the same dose of visible light).

For a constant, large dose of visible light the survival curve of the complexes is similar to a three-hit curve (Fig. 4). Among the reactivated phages no mutants are found.

(c) Photorestitution of the complexes (UV'd phage-UV'd bacterium).

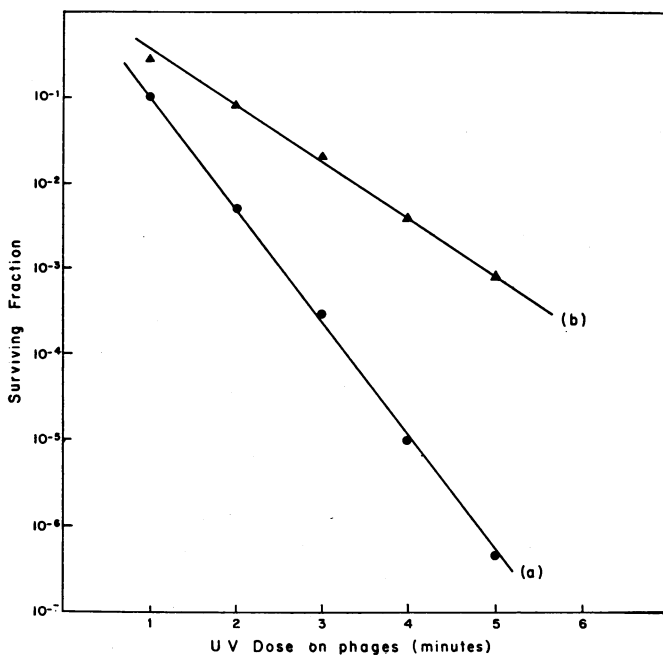


FIGURE 4

Photoreactivation of the complexes (UV'd phage-bacterium). Semilogarithmic plot of the surviving fraction of UV'd λ plated (a) on non-irradiated bacteria in the dark, (b) on non-irradiated bacteria and then illuminated for 3 hours at room temperature under the fluorescent lamps, (c) on 60-sec. UV irradiated bacteria in the dark, (d) on 60-sec. UV irradiated bacteria and then illuminated as in (b). The coincidence of curves (b) and (c) is fortuitous for they are separated when other bacterial strains are used for plating.

—Illumination with visible light of these complexes produces restoration and the survival curve is again a three-hit curve (Fig. 4). The maximum reactivation obtainable following both UV treatment of the bacteria and photorestitution seems to be the same for all the bacterial strains. Among the photorestored complexes the same type of mutants are found as before photorestitution but their proportion has decreased. The fact that, in

these conditions, some of the UV'd bacteria are restored and thus rendered unable to reactivate the phages, may be responsible for this decrease.

Discussion and Conclusions.—When temperate phages infect a sensitive bacterium they may provoke either a lytic or a lysogenic response. In the case of the lytic response the phage goes into the vegetative state, multiplies rapidly and the cell lyses after a definite latent period liberating newly formed particles. In the case of the lysogenic response the phage is reduced to the prophage state and the bacterial cell, by repeated division, gives rise to a clone whose bacteria all carry the prophage, that is the ability to lyse with liberation of phages upon the application of the right sort of inducing stimulus. This induction of lysogenic bacteria to lyse is caused in K12 (λ) by treatment with UV (15 sec. of irradiation produce induction in 99.9% of the cells), with x-rays or with nitrogen mustard.

After UV irradiation the phages are called inactive because they have lost their ability to multiply and thus to form plaques when plated on sensitive bacteria. If the phages are temperate, this inactivation means that they are no longer able to provoke the lytic response of the bacteria. As UV'd temperate phages do not kill the bacteria it is possible to imagine that they are provoking the lysogenic rather than the lytic response in the bacteria they have infected. If this were true, UV'd temperate phages plated on sensitive bacteria and, *at any time later*, given a further *inducing* dose of UV should show a larger number of survivors than if they had not been induced.

This is what happens for λ except that an hour after adsorption of the phages to the bacteria the "inducibility" has disappeared. Thus the UV'd phages did not form true lysogenic complexes. Another experiment points to the same conclusion: the virulent mutant of λ , which never forms lysogenics, also shows an increase in the number of plaques after irradiation of the complexes (UV'd virulent mutant-bacterium).

Thus the increase in the number of survivors by UV irradiation of the complexes (UV'd phage-bacterium) or by irradiation of the bacteria separately is a new type of reactivation. We shall call it UV restoration (UVR) in view of the fact that most of the experiments reported here were made with UV. It is possible that further experiments with x-rays and nitrogen mustard may show what is common to the action of these agents and allow a better name to be chosen to describe this reactivation.

The experiments have shown that the UV'd phages, in order to be UVR'd, must be adsorbed on UV'd bacteria. It is thus the bacteria that are reactivating and not a substance they might have released after irradiation.

The bacteria reactivate the UV'd phages under the following conditions: (1) When they have been treated with x-rays, with nitrogen mustard, or with UV. These three agents are inductors of lysogenic bacteria, they are mutagenic, and they disrupt the nuclear apparatus of the bacteria. It is

not known if these different actions are one aspect of the same cause, or which of these effects is responsible for reactivation. (2) The bacteria conserve the reactivating property for a long time when they are kept in buffer at 4°C. (3) The effect of UV on the bacteria, making them reactivating, can be reversed by exposure to visible light. (4) The reactivation as a function of the dose of UV given to the bacteria can be described by assuming that a proportion of the UV'd bacteria is in the reactivating state. This proportion is independent of the UV dose given to the phages. It is very approximately equal to the proportion of bacteria killed as colony formers.

Let us now sum up our findings concerning the properties of the reactivated phages: (1) The phage progeny liberated by a UV'd bacterium infected by a UV'd phage, consists of normal (except for mutations) viable phages which plate with equal efficiencies on both normal and irradiated bacteria. (2) UV'd phages plated on normal bacteria can be photoreactivated (PhR). If after the PhR the complexes are irradiated with UV, a further reactivation takes place. This is also true when UV irradiation has been given prior to illumination. This shows that the two classes of phages reactivable by visible light and by UV'd bacteria, respectively, are not identical. (3) After the double action of PhR and UVR a certain proportion of the UV'd phages remain inactivated, showing that certain UV damages cannot be repaired either by light or UV. (4) Mutants appear among the UVR'd phages. The action of UV on *both* the phages and the bacteria is necessary for their appearance. The mutation affects the entire progeny of the primary UV'd phage particle.

These facts can be accounted for by the following assumptions: UV irradiation produces four different types of lesions or damages in the phages. Type 1 lesions are not repairable by either UVR or PhR. Type 2 are repairable by either, type 3 by PhR only, and type 4 by UVR only.

Any one phage particle may have suffered damages of several of these types as a result of exposure to UV. A small fraction of the type 4 damages and *only of the damages of this type* are associated with mutations.

Our experiments do not tell us what is the specific role played by the action of the UV on the phages and on the bacteria, respectively. They rather emphasize that *both* actions are necessary. This double causation is very obvious in the present case. It raises the question whether mutagenesis in other organisms might not involve a similar double causation.

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¹ Bertani, G., and Weigle, J., *J. Bact.*, **65**, 113 (1953).

² Adams, M. H., in *Methods in Medical Research*, Chicago, 1950.

³ Dulbecco, R., *J. Bact.*, **59**, 329 (1950).

⁴ Jacob, F., personal communication.

⁵ We wish to thank Ciba S.A., Basel, Switzerland, who put at our disposal the chlorohydrate of methyl bis- β -chlorethylamine, called Dichlorene, used in these experiments.

⁶ See Lwoff, A., *Ann. Inst. Past.*, **84**, 225 (1953).

⁷ Hayes, W., *J. Gen. Microb.*, **8**, 72 (1953).

⁸ Cavalli, L. L., Lederberg, J., and Lederberg, E. M., *Ibid.*, **8**, 89 (1953).

RELATIONS ON ITERATED REDUCED POWERS*

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In this note we present the generalization of the relations on iterated squares¹ to the case of iterated cyclic reduced powers of arbitrary prime period p . As in the case $p = 2$, the new relations are used to solve some particular problems.

Throughout this paper we will use the definitions and notation recently introduced by Steenrod.²

1. For any complex K and odd prime p , the cyclic reduced power operations are homomorphism \mathcal{O}^s , ($s = 0, 1, \dots$),

$$\mathcal{O}^s: H^q(K; \mathbb{Z}_p) \rightarrow H^{q+2s(p-1)}(K; \mathbb{Z}_p)$$

They satisfy the following properties: $\mathcal{O}^s f^* = f^* \mathcal{O}^s$, where f is a map of one complex into another; $\mathcal{O}^0 = \text{identity}$; if $q = \dim u$ is even, $\mathcal{O}^{q/2} u = u^p$ (in cup-product sense); $\mathcal{O}^s u = 0$ when $s > q/2$.

As in the case of squares, an *iterated* cyclic reduced power is a composition of two or more of the \mathcal{O}^s , e.g., $\mathcal{O}^r \mathcal{O}^s \mathcal{O}^t$.

Let δ^* be the coboundary operator associated with the exact coefficient sequence $0 \rightarrow \mathbb{Z} \rightarrow \mathbb{Z} \rightarrow \mathbb{Z}_p \rightarrow 0$. Our main result is the following

THEOREM 1.1 For all $0 \leq r < sp$ the iterated cyclic reduced powers satisfy the following set of relations³

$$(1.2) \quad \mathcal{O}^r \mathcal{O}^s = \sum_{i=0}^{[r/p]} (-1)^{r+i} \binom{(s-i)(p-1)-1}{r-ip} \mathcal{O}^{r+s-i} \mathcal{O}^i,$$

$$(1.3) \quad \mathcal{O}^r \delta^* \mathcal{O}^s = \sum_{i=0}^{[r/p]} (-1)^{r+i} \binom{(s-i)(p-1)}{r-ip} \delta^* \mathcal{O}^{r+s-i} \mathcal{O}^i + \sum_{i=0}^{[(r-1)/p]} (-1)^{r+i+1} \binom{(s-i)(p-1)-1}{r-ip-1} \mathcal{O}^{r+s-i} \delta^* \mathcal{O}^i, \quad (\text{mod } p),$$